REMARKS

This Amendment and Response document is being submitted in response to the final Office Action dated March 19, 2009 where Claims 1, 3, 5-10, 19 and 20 are pending. Applicants have amended Claim 1. This amendment has been made to place to claims in better form for examination and to further obviate the 35 U.S.C 102(b) and 103(a) rejections. It is believed that none of these amendments constitute new matter.

Rejections Under 35 U.S.C. § 112 Withdrawn

The Examiner has withdrawn the previous rejection of Claims 3 and 19 under 35 U.S.C. § 112, second paragraph, based on the claim amendments submitted in the previous response.

Rejections Under 35 U.S.C. § 102(b) Hu

The Examiner has maintained the rejection of Claims 1, 3, 9-10 and 19 under 35 U.S.C. § 102(b) as being anticipated by Hu (U.S. Patent No. 5,939,251). Applicants have amended Claim 1. In addition, Applicants respectfully traverse this rejection.

A *prima facie* case of anticipation requires that a single publication teach, either expressly or inherently, each and every element or limitation of the claim, including any functional limitations. M.P.E.P. § 2131. According to the Examiner, Hu teaches a method of performing *in situ* PCR within a solid support having multiple compartments, wherein the cells are directly fixed on the solid support. The Examiner has also specifically pointed out that Hu teaches a method comprising: determining whether amplified nucleic acids in a PCR solution contains the target nucleic acid (col. 6, lines 25-35).

Applicants have amended Claim 1 to clarify that the target nucleic acid is exposed (outside of the cell sample) to the sample surface by a pre-treatment step, which precedes the nucleic acid amplification step. Example 2 in the specification clearly describes pre-treatment of samples (nucleic acid exposing step) (pgs. 43-44). In this example, the pre-treatment step involves an enzyme treatment method that lyses the cells, which is known by those skilled in the art to dissolve the cell membrane and thus expose, to the outside of the cell, the nucleic acids (as well as proteins and lipids) that were within the cell to allow accessibility to these cellular

components (i.e. allow for primers and nucleic acid polymerases involved in the amplification step to reach the target nucleic acids, pg 18 of the specification). Applicants do note that detergent pre-treatment methods and heat pre-treatment methods (and combination) are also known to those skilled in the art and not limited to an enzyme pre-treatment method (see pgs. 18-19 for discussion on the Nucleic Acid Exposing Step). The use of the thermal cycler in this step, is well known by those skilled in the art to heat the sample (activate the enzyme lyses reaction) and then used to deactivate the enzyme lyses reaction when increasing the temperature for a brief time. The amplification (via Nested-PCR in this example) of the nucleic acids occur after the pre-treatment step (pgs. 43-47) and thus the heat from the PCR amplification step is not the same as the heat described in the pre-treatment step. Applicants therefore contend that with regard to Claim 3, the heat from the PCR does not encompass the heat from a pre-treatment step (exposing step). In addition, the Applicants contend that Hu does not teach or suggest the method as now claimed in Claim 1 (see below for discussion) and therefore Claims 1, 3, 9-10 and 19 are not anticipated by Hu.

The Applicants contend that *in situ* PCR (as taught by Hu), is commonly known by those skilled in the art to be a PCR reaction that actually takes place inside the cell on a slide and is performed on fixed tissue or cells. The *in situ* PCR method is an improvement of an *in situ* hybridization (ISH) that was developed to detect a nucleic acid amplified in a cell while maintaining the conformation of the cell (see, e.g., column 2, lines 5-8, and column 6, lines 25-27 of Hu). Determining the existence of the target nucleic acid in a PCR solution is claimed in Claim 1 of the present invention and thus the amplified gene to be detected exists extracellularly, which Hu does not teach nor suggest. Hu teaches a method for performing a molecular biological reaction such as an *in situ* polymerase chain reaction (PCR) and *in situ* hybridization (ISH) (column 1, line 14; column 6, line 22 of Hu). The *in situ* PCR method of Hu is fundamentally different than the claimed invention in that Hu teaches a method used for detecting intracellular localization of a target nucleic acid (i.e., detecting a target nucleic acid existing in a cell or tissue). Therefore, the Applicants contend that Hu does not anticipate the invention as claimed.

Additionally, Applicants would like to point out that in the case of *in situ* PCR, an amplified gene to be detected could not exist extracellularly because of a washing step after amplification of a gene *in situ* (see for example, Villeponteau et al. U.S. Patent No.5,776,679, column 42, lines 41-42). Furthermore, because the washing step after amplification *in situ* is important in *in situ* PCR, one skilled in the art would know that the washing step cannot easily be replaced with another step. Needless to say, one skilled in the art cannot delete such an important step of washing a cell after amplification of a gene *in situ* and this the amplified gene to be detected could not exist extracellularly as taught in the present invention.

In regards to Claim 19, the Examiner states that Hu teaches the slide as able to fit within a thermal cycling apparatus (Office Action page 3). Applicants assert that the slide described by Hu is designed specifically for carrying out a thermal cycling operation to perform an *in situ* PCR amplification process (colm 6, lines 18-24 of Hu) and not for the PCR amplification process as claimed in the instant invention and thus does not anticipate Claim 19.

Thus, for the reasons discussed above, Hu does not disclose or suggest each and every element of Claims 1, 3, 9-10 and 19. Accordingly, Hu does not anticipate Claims 1, 3, 9-10 and 19, and Applicants respectfully request that this rejection be withdrawn.

Rejections Under 35 U.S.C. § 103(a) Hu in view of Villeponteau et al.

The Examiner has maintained the rejection of Claims 5 and 20 under 35 U.S.C. § 103(a) as being unpatentable over Hu (U.S. Patent No. 5,939,251) in view of Villeponteau et al. (U.S. Patent No. 5,776,679). The Examiner has stated that Hu does not expressly teach the labeling of nucleic acids during PCR or detection of PCR products through electrophoresis. However, the Examiner states that Villeponteau et al., teaches labeling nucleic acids during *in situ* PCR through the incorporation of labeled nucleotides (column 42, line 50-65). Thus it would be *prima facie* obvious to a skilled artisan to incorporate labeled nucleotides into the *in situ* PCR of Hu. In addition, the Examiner has also stated in regards to Claim 20, that the detection of PCR products through electrophoresis was well known as a standard method of PCR product detection and that Villeponteau et al teaches such an electrophoresis method (column 31, example 3).

Applicants have amended Claim 1 in which Claims 5 and 20 depend upon. The disclosure of Villeponteau et al. does not overcome the deficiencies of Hu as discussed above and thus neither Hu nor Villeponteau et al., whether considered alone or in combination teach each and every element of Claims 5 and 20 which depend on amended Claim 1.

A *prima facie* case of obviousness has three distinct requirements. First, the references must teach or suggest every claim element. M.P.E.P. §§ 2142 and 2143.03. Second, there must be a motivation to modify or combine the teachings of the cited references. M.P.E.P. §§ 2143 and 2143.01. Third, there must be a reasonable expectation of success in performing the modified or combined teachings of the references. M.P.E.P. § 2143.02.

The Applicants contend that neither Hu nor Villeponteau et al. teach or suggest the step of determining whether the amplified nucleic acids in the PCR solution contain the exposed target nucleic acid. As mentioned above, Hu teaches an *in situ* PCR method which is not the same as the detection method of the invention as claimed (due to amplification of the target within the cell or tissue and the important washing step, for example).

Therefore, even combining the teachings of Hu and Villeponteau et al., one does not arrive at the invention recited in Claim 5 or Claim 20. Because none of these references, alone or combined, teach each and every element of Claim 5 and Claim 20, Applicants submit that Claim 5 and Claim 20 are patentable over the cited references. The Applicants thus respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

Rejections Under 35 U.S.C. § 103(a) Hu in view of Villeponteau et al. in further view of Stapleton et al.

The Examiner has maintained the rejection of Claims 6-8 under 35 U.S.C. § 103(a) as being unpatentable over Hu (U.S. Patent No. 5,939,251) in view of Villeponteau et al. (U.S. Patent No. 5,776,679) as applied to Claim 5 and in further view of Stapleton et al. (U.S. Patent No. 6,103,192). The Examiner has stated that the previously applied references do not expressly teach the detection of labeled PCR products through hybridization to an immobilized probe in microarray format. The Examiner states that Stapleton et al teaches a method wherein various

biological specimens are collected, dried, transported, stored and processed on matrixes which adhere cells and viruses. Additionally as concluded by the Examiner, Stapleton states that "such a detection system eliminates the need for gel electrophoresis,. . ., and allows for multiple oligonucleotide sequences at different array positions to be analyzed in the same detection reaction". Applicants have amended Claim 1 in which Claims 6-8 depend upon.

Even if the combination of Hu and Villeponteau et al. and Stapleton et al. is made as suggested by the Examiner, none of the references alone, nor the combination of references discloses the claimed invention, namely the claimed nucleic acid detection method that includes the step of determining whether the amplified nucleic acids in the PCR solution contain the exposed target nucleic acid. More specifically, the *in situ* PCR method of Hu is a method used for detecting intracellular localization of a target nucleic acid (i.e., detecting a target nucleic acid existing in a cell or tissue), while as stated in Claim 1, determining the existence of the target nucleic acid in a PCR solution is a claimed feature and thus the amplified gene to be detected exists extracellularly. Thus, it has not been established that the references, considered alone or in combination teach or suggest the invention as claimed.

In order to support a prima facie case of obviouness, a combination of references must teach each and every one of the claimed elements. Since the combination of Hu and Villeponteau et al. and Stapleton et al. does not teach each of the elements of Claims 6-8, withdrawal of the rejection is respectfully requested.

Closing Remarks

Applicant believes that the pending claims are in condition for allowance. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-1970, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-1970.

Respectfully submitted,

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